

BREEDING SYSTEMS OF TWO ENDEMIC RAINFOREST SPECIES IN SOUTHERN CHILE: *AMOMYRTUS MELI* (PHIL.) LEGR. ET KAUS. (MYRTACEAE) AND *LUZURIAGA POLYPHYLLA* (HOOK.) MACBR. (PHILESIACEAE)

SISTEMA REPRODUCTIVO DE DOS ESPECIES ENDEMICAS DEL BOSQUE LLUVIOSO DEL SUR DE CHILE: *AMOMYRTUS MELI* (PHIL.) LEGR. ET KAUS. (MYRTACEAE) Y *LUZURIAGA POLYPHYLLA* (HOOK.) MACBR. (PHILESIACEAE)

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ABSTRACT

RESUMEN

Experimental hand self- and cross-pollinations, spontaneous selfing trials and emasculation tests to detect compatibility status, selfing capacity and agamospermy, along with natural fruiting levels were undertaken in the rainforests of Chiloé, 42°S on *Amomyrtus meli* (Myrtaceae), endemic to Chile and belonging to a genus endemic to temperate southern South America, and on *Luzuriaga polyphylla* (Philesiaceae), endemic to Chile and belonging to a small genus of Gondwanan distribution. *Amomyrtus meli* is genetically self-compatible and non-agamospermous. Natural pollination fruit set was statistically indistinguishable from hand cross-pollination and hand self-pollination fruit set. This is the second record of genetic self-compatibility in *Amomyrtus*, confirming that the entire genus is self-compatible. Self-compatibility in *Amomyrtus* contrasts with presence of genetic self-incompatibility in other genera of Myrtaceae (*Luma* and *Myrceugenia*) in the temperate rainforest of southern South America. *Luzuriaga polyphylla* is strongly self-incompatible and non-agamospermous. Natural pollination fruit set was statistically indistinguishable from hand cross-pollination fruit set, indicating efficient pollination in the rain forest of Chiloé. Self-incompatibility in *L. polyphylla* constitutes a second report of this breeding system among South American species of the genus, where *L. radicans* has also been reported as self-incompatible, and adds another species to the growing list of obligately outbred species among woody and semi-woody elements in the rainforest flora of southern South America.

KEYWORDS: *Amomyrtus meli*, *Luzuriaga polyphylla*, Myrtaceae, Philesiaceae, genetic self-compatibility, genetic self-incompatibility, endemic species of Chile, rainforest, Chile.

Se dan a conocer los resultados de pruebas de autopolinización manual, polinización cruzada manual, autopolinización automática y emasculación, para la detección del tipo de compatibilidad, autogamia y agamospermia en *Amomyrtus meli* (Myrtaceae), especie del bosque lluvioso, endémica de Chile, y perteneciente al género sudamericano austral endémico, y en *Luzuriaga polyphylla* (Philesiaceae), especie endémica a Chile y perteneciente a un género de distribución Gondwanica. *Amomyrtus meli* es genéticamente autocompatible. La producción de frutos mediante la polinización natural es estadísticamente indistinguible del nivel de fructificación obtenido por polinización cruzada manual y autopolinización manual. El registro de autocompatibilidad genética en *A. meli* constituye el segundo de este sistema de reproducción para el género, confirmando que la autocompatibilidad es característica a nivel genérico. La autocompatibilidad genética en el género *Amomyrtus* contrasta con la presencia de autoincompatibilidad genética en otros géneros de la familia Myrtaceae (*Luma* and *Myrceugenia*) del bosque lluvioso del sur de Sudamérica. *Luzuriaga polyphylla* es altamente autoincompatible y no presenta agamospermia. La producción de frutos mediante la polinización natural es estadísticamente indistinguible al nivel de fructificación obtenido mediante la polinización manual cruzada, indicando un sistema de polinización muy eficiente en esta especie en los bosques de Chiloé. La presencia de autoincompatibilidad genética en *L. polyphylla* constituye el segundo registro de este sistema de reproducción entre las especies sudamericanas del género, donde *L. radicans* fue recientemente documentada como genéticamente autoincompatible. Con el presente trabajo se agrega otra especie genéticamente incompatible a la lista creciente de especies exógamas entre los elementos leñosos y semi leñosos de los bosques lluviosos del sur de Sudamérica.

PALABRAS CLAVES: *Amomyrtus meli*, *Luzuriaga polyphylla*, Myrtaceae, Philesiaceae, autocompatibilidad genética, autoincompatibilidad genética, especies endémicas de Chile, bosque lluvioso, Chile.

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INTRODUCTION

The question as to whether genetic self-incompatibility is basal to the angiosperms and or has evolved on several different occasions within different angiosperm lineages is still an open one (Weller *et al.*, 1995). The answer to this crucial question in angiosperm evolution requires better knowledge of the distribution of self-incompatibility at the generic and familiar levels. Particularly critical, are studies in woody species, vines and long-lived perennial species where self-incompatibility tends to be more frequent (Arroyo & Squeo, 1990 a and b; Arroyo & Usler, 1993). However, because species in these life forms must usually be studied in field populations, they tend to garner less attention. In this paper we report the results of experimental tests to detect genetic self-incompatibility in *Amomyrtus meli* (Phil.) Legr. et Kaus. (Myrtaceae) and *Luzuriaga polyphylla* (Hook.) Macbr. (Philesiaceae) inhabiting the rainforests of Chiloé, Chile. In both of these two small genera, other species have already been tested for self-incompatibility (Riveros *et al.*, 1996).

Amomyrtus meli (Phil.) Legr. et Kaus. is a large rainforest tree to 20 m tall occurring from Arauco to Chiloé. The genus *Amomyrtus* (Burret) Legr. et Kaus. (Myrtaceae), comprising two species, is endemic to southern South America (Arroyo *et al.*, 1996). *Amomyrtus luma* (Mol.) Legr. et Kaus., the second species, is a shrub or tree occurring in humid forest habitats from Maule to Aysen in Chile, and Neuquén to Chubut in the Andes of Argentina. Both species of *Amomyrtus* have large white flowers with copious pollen, and fleshy black fruits (Landrum, 1988). Flowers of *A. luma* are known to be visited by Hymenoptera (Halictidae) and Diptera (Syrphidae) (Riveros *et al.*, 1991). Although the ovaries of these species contain numerous ovules, mature fruits normally contain 1-3 large seeds, 4-6 mm long, these being larger in *A. meli*. Riveros *et al.* (1996) recently reported the presence genetic self-compatibility in *Amomyrtus luma* based on work in the Valdivian rainforest, 40°S, Chile. It is of special interest, thus, to determine whether self-incompatibility characterizes *A. meli*. *Amomyrtus meli*, unlike the *A. luma*, is strictly endemic to Chile.

Luzuriaga polyphylla (Hook.) Macbr. is a high-climbing semi-woody epiphytic species

with large solitary flowers containing small amounts of nectar and with fleshy fruits. *Luzuriaga* is a small monocotyledonous genus of Gondwanan distribution containing 4 species (Rodríguez & Marticorena, 1987; Arroyo & Leuenberger, 1988; Arroyo *et al.*, 1996). *Luzuriaga polyphylla* is endemic to Chile where it occurs from 0-800 m elevation in Regions VIII to IX (Rodríguez & Marticorena, 1987), being especially abundant in rainforest on the Coast Range from Valdivia south. Riveros *et al.* (1996) recently reported genetic self-incompatibility in *Luzuriaga radicans* based on work in the Valdivian rainforest, 40°S, Chile. Nothing is known about the breeding system of *L. parviflora* (Hook. f.) R. et P. distributed in New Zealand (Rodríguez & Marticorena, 1987).

MATERIALS AND METHODS

Tests on *Amomyrtus meli* were conducted during November, 1997 at the Estación Biológica "Senda Darwin", Chiloé (42°S). Tests on *Luzuriaga polyphylla* were conducted over the period October 1997- January 1998 at the same locality. Here both species occur in perhumid rainforest zone (Arroyo *et al.*, 1996) along with *Podocarpus nubigena*, *Saxegothaea conspicua*, *Weinmannia trichosperma*, *Caldcluvia paniculata*, *Myrceugenia planipes* and *Tepualia stipularis* as the main tree species. Opaque-white-flowered *Luzuriaga polyphylla* grows on living trunks about 1-2 m from ground level. Annual precipitation at Pudeto, located close to Senda Darwin is 1.809 mm; mean annual temperature is 11°C (Hajek & Di Castri, 1975).

To investigate the breeding system, hand self-pollinations, hand cross-pollinations, spontaneous selfing trials and emasculations without further pollination were conducted on plants growing in the field previously bagged at the flower bud stage in white organza pollination bags. Each test was conducted on several different individuals growing at the same location. Pollinations on *Luzuriaga polyphylla* were spread over a period of 15 days; those on *Amomyrtus meli* over 20 days. Over these same periods, to evaluate the efficiency of the pollination system, additional unbagged flowers on both species were marked and assessed for fruit set so as to compare the level of fruiting under natural conditions of pollination

to that obtained under hand cross-pollination. For self-pollination, pollen from the same flower or from other flowers on the same individual was used. For cross-pollination, fresh pollen was collected from individuals located distant from the test plant, and transported immediately to the receptive stigmas of bagged flowers. Each flower was pollinated on three separate occasions so as to increase the likelihood of encountering the stigmas at their maximum period of receptivity and of obtaining physiologically maximal fruit and seed sets. All pollinations were carried in the early afternoon when temperatures were warmer and the flowers were fully open. Following marking, all manipulated flowers were immediately rebagged. Complete fruit maturation in *Luzuriaga polyphylla* in Chiloé requires around 8-9 months (Smith-Ramírez & Armesto, 1994). Long fruit maturation periods have also been described in *L. marginata* (Arroyo & Leuenberger, 1988) and would appear to be characteristic of the genus. In order to avoid loss of fruits in the experimental crosses and other tests due to autumn and winter storm damage, we harvested fruits of this species in late summer as they began to mature. Fruit maturation in *Amomyrtus meli* occurs over the period December to April (Smith-Ramírez & Armesto, 1994). For our experiments, fruits were harvested in April, 1998. In both species pollination bags were retained on the plants until the time of fruit collection, so as to prevent fruit loss through the action of the biotic dispersal agents (Armesto *et al.*, 1987) and facilitate capture of any mature fruits that potentially could have fallen by the time of fruit harvest. Fruits of *Luzuriaga polyphylla* tend to fall easily with any disturbance.

RESULTS AND DISCUSSION

Under hand self-pollination 13.5% of the flowers of *Amomyrtus meli* produced fruits, in comparison with 24.3% under hand cross-pollination. In the spontaneously selfing trials, 10.5% of the tested flowers produced fruits (Table I). Cross-pollination and self-pollination fruit set for *A. meli* ($G_{adj} = 2.234$; NS) were not significantly different, as was the case for self-pollination and spontaneous self-pollination ($G_{adj} = 0.269$; NS). Cross-pollination fruit set was significantly higher

than spontaneous self-pollination fruit set ($G_{adj} = 5.415$; $p < 0.05$). The ISI (measure of degree of self-incompatibility fluctuating between 0 and 1; 0 = full self-incompatibility; 1 = full compatibility - see Ruiz & Arroyo, 1978) for *A. meli* is 0.50, which is well above the value of 0.2 used by convention to differentiate between self-incompatible and self-compatible species (c.f. Arroyo & Squeo, 1990b). *Amomyrtus meli* thus is classed as a self-compatible species, although clearly, the level of compatibility is only moderate.

Only 3.5% of the hand self-pollinated flowers of *Luzuriaga polyphylla* produced fruit (Table II). In the spontaneously selfing trials, no flowers produced fruits, as was the case for emasculated flowers (Table II). In contrast, 58.5% of hand pollinated cross-pollinated flowers produced fruits, a proportion that is much higher than with hand self-pollination. These results give an ISI value of 0.026, which is well below the cut-off value of 0.2 and allow the conclusion of a high level of genetic self-incompatibility in *Luzuriaga polyphylla*.

Natural fruit production in *Amomyrtus meli* was moderate (Table III), but not significantly different from that in any of the experimental treatments ($G_{adj} = 1.696$; NS; (spontaneous selfing); $G_{adj} = 0.519$; NS (hand self-pollination); $G_{adj} = 0.413$, NS (hand cross pollination)). Therefore the fruits produced under natural conditions of pollination in *Amomyrtus meli* could have been derived from spontaneous intra-flower self-pollination or geitonogamy (self-pollination as a result of pollen transference among flowers of the same individual) or cross-pollination. Mostly likely natural pollination fruit set is derived from all types of pollination.

Natural fruit production in unbagged flowers of *Luzuriaga polyphylla* was surprisingly high (Table IV) for a strongly self-incompatible species and not significantly different ($G_{adj} = 1.46$; NS) from that obtained under hand-pollination in which all stigmas were artificially pollinated, suggesting high efficiency of the natural pollination system. Seed number per fruit was somewhat higher in naturally pollinated flowers than in hand cross-pollinated flowers (t (d.f. = 80) = 5.598; $p < 0.001$) (Table IV). This last situation is commonly encountered in field pollination tests (c.f. Arroyo & Squeo, 1990a) where, in spite of repeated pollinations, it is difficult to

assure the exact timing of maximum stigma receptivity. As an aside, it should be noted that mature fruits of *L. polyphylla* are greenish at maturity in Chiloé (Armesto *et al.*, 1987). Hoffmann (1982) illustrates the fruits of *L. polyphylla* as greenish-yellow. Rodríguez & Marticorena (1987) describe the fruits of *L. polyphylla* as reddish-orange. Evidently more work is needed to define fruit colours in the South American species of *Luzuriaga*. The high level of genetic self-incompatibility demonstrated in *Luzuriaga polyphylla* highlights its dependence upon external pollinating agents. Riveros *et al.* (1991) reported that flowers of *L. radicans* are visited by halictid bees in Parque Nacional Puyehue, 40°S, and flowers of *L. marginata*, a species closely related to *L. polyphylla* (Rodríguez & Marticorena, 1987), are reported to be sweetly fragrant (Arroyo & Leuenberger, 1988), also suggesting bee pollination. Unfortunately nothing is presently known about the pollination biology of *L. polyphylla*. We failed to observe pollinator activity on *L. polyphylla* while carrying out our crossing experiments. However, causal visits by *Bombus dahlbomi* to the very similar flowers of *L. radicans* were observed. *Bombus dahlbomi* is a common large bumblebee in the forests of Chiloé. Undoubtedly a detailed study of the pollination mechanisms of the three Chilean species, taking their wide latitudinal distributions into account, would prove very interesting.

The results reported here in conjunction with earlier work by Riveros *et al.* (1996) on *Amyrtus luma* confirm that all extant species of the genus *Amyrtus* are self-compatible. This is particularly interesting, since self-incompatibility has been found in *Myrceugenia* and *Luma* (Riveros *et al.*, 1996). Little is known about the relative phylogenetic positions of these three genera of Myrtaceae. The presence of self-compatibility in *Amyrtus* could be indicative of a derived condition. The majority of woody species in the southern South American temperate rain forest are dioecious or genetically self-incompatible, as indeed is the case in other woody formations in general in Chile (Arroyo & Uslar, 1993; Riveros *et al.*, 1995, 1996; Castor *et al.*, 1996). *Amyrtus* at the generic and specific level, is clearly an exception.

Demonstration of self-incompatibility in *Luzuriaga polyphylla*, a species with some degree of woodiness, is consistent with the strong

representation of self-incompatibility and other outcrossing breeding systems such as dioecism among woody species in temperate forests in Chile (Arroyo & Uslar, 1993; Riveros *et al.*, 1995, 1996; Castor *et al.*, 1996) and adds another species to the long list of self-incompatible species in southern South American temperate rainforests. The presence of self-incompatibility in two South American species of *Luzuriaga* raises the question about the remaining two species of the genus, and especially New Zealand *L. parviflora*. In view of the disjunct distribution of the genus in New Zealand and southern South America, information on the breeding system of the latter species would be particularly welcome. Interestingly, and in contrast with what has been demonstrated in the Chilean flora (e.g. Arroyo & Squeo, 1990b; Arroyo & Uslar, 1993; Riveros *et al.*, 1995, 1996) self-incompatibility is known for a very limited number of species in the New Zealand flora, although as Webb & Kelly (1993) point out, the sample size for New Zealand is perhaps still too small for any convincing conclusions at this stage.

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TABLE I. Results of controlled hand pollinations, spontaneous selfing trials and emasculation tests in *Anomyrtus meli* (Myrtaceae) in temperate rainforest in Chiloé.

Test	Plants	Flowers	Fruits			Mean Seeds/flower crossed
			N	Percent	Seed	
Self-pollinated	4	52	7	13.5	7	0.13
Cross-pollinated	5	70	17	24.3	19	0.27
Spontaneously self-pollinated	5	95	10	10.5	12	0.13
Emasculated	5	51	0	0	-	-

TABLE II. Results of controlled hand pollinations, spontaneous selfing trials and emasculation tests in *Luzuriaga polyphylla* (Philesiaceae) in temperate rainforest in Chiloé.

Test	Plants	Flowers	Fruits			Mean Seeds/flower crossed
			N	Percent	Seed	
Self-pollinated	7	57	2	3.5	6	0.11
Cross-pollinated	3	53	31	58.5	225	4.25
Spontaneously self-pollinated	8	69	0	0	-	-
Emasculated	3	32	0	0	-	-

TABLE III. Comparison of natural fruit and seed set compared with controlled cross-pollination fruit and seed set in *Amyrtyus meli* in temperate rainforest in Chiloé. Cross pollination fruit set from Table I.

Test	Plants	Flowers	Fruits		Mean seeds per fruit (SE)
			N	%	
Cross-pollination	5	70	17	24.3	1.1±0.08
Natural pollination	4	42	8	19.1	1.0±0

TABLE IV. Comparison of natural fruit and seed set compared with controlled cross-pollination fruit and seed set in *Luzuriaga polyphylla* in temperate rainforest in Chiloé. Cross pollination fruit set from Table II.

Test	Plants	Flowers	Fruits		Mean seeds per fruit (SE)
			N	%	
Cross-pollination	3	53	31	58.5	7.26 (0.23)
Natural pollination	8	74	51	68.9	8.69 (0.33)

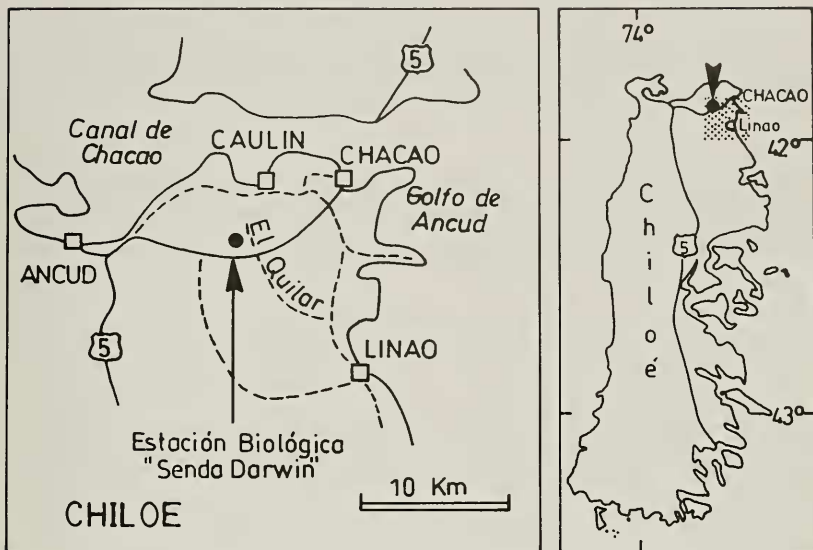


FIGURE 1. Location of "Senda Darwin" study site on the island of Chiloé.

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